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RESEARCH ARTICLE

Pilot progress in DNA isolation and amplification from the material stored at the LWS herbarium

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Abstract

The isolation of DNA from the herbarium specimens deposited at the LWS herbarium (State Museum of Natural History of the NAS of Ukraine, Lviv, Ukraine) has been tested using the column-based protocol. The isolated DNA has been amplified using different nuclear and plastid primers. The yield of obtained total DNA showed no significant dependence from the year of collection and plant family of studied specimens. In general, the obtained DNA of LWS specimens had medium yield (mean – 56.47 ng/μL) but relatively low purity (mean 260/230 value – 0.85 units and mean 260/280 value – 1.66 units). The success of DNA amplification for old herbarium material varied from 12.5 % to 91.1 % depending on applied primers. The *trnL* P6 Loop primers demonstrated the best performance (91.1 % successful amplification), but due to short resulted DNA fragments, it was not possible to purify the product for further processing. UniPlant primers performed the worst, and only 12.5 % of samples taken from the LWS herbarium (excluding controls) were successfully amplified. In general, nuclear primers, except for UniPlant, demonstrated a better success rate (mean – 31.5 %) during the work with samples taken from the LWS herbarium. Meanwhile, the plastid primers, except for *trnL* P6 Loop, showed slightly lower amplification success (mean – 26.8 %).

Keywords: herbarium specimens, plant DNA barcoding, DNA extraction methods, degraded DNA, LWS herbarium

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Introduction

Despite the development of modern approaches to biodiversity data gathering by community science, herbarium collections remain a key point for different studies, including biogeographic, phylogenetic, and

taxonomic (e.g., Nualart et al., 2017; Besnard et al., 2018; Martin et al., 2018; Lang et al., 2019; Rosche et al., 2022, 2025). It was shown that herbarium collections outperform community science platforms (i.e., iNaturalist) by providing the data with lower spatial, taxonomic, phylogenetic, and functional bias (Daru &

Rodriguez, 2023; Eckert et al., 2024). Providing well-documented specimens preserved for decades or even hundreds of years, the herbaria have attracted the attention of molecular biologists for many years (Savolainen et al., 1995; Särkinen et al., 2012; Bakker et al., 2020; Bieker & Martin, 2018; McAssey et al., 2023). However, the DNA in long-stored herbarium material is often highly degraded (Adams & Sharma, 2010; Staats et al., 2011). It was shown that DNA degradation and fragmentation tend to increase over time, resulting in shorter lengths of reads and extensive accumulation of cytosine-to-thymine substitutions (Weiß et al., 2016; Quatela et al., 2023). Forrest et al. (2019) demonstrated considerable variation in the read length for *Begonia* L. depending on the preservation method. They suggested that such differences can be even higher at higher taxonomic levels. Despite all mentioned problems, the undoubted value of the material stored at the herbaria prompts the search for special techniques for DNA isolation and amplification, as well as the reconstruction of short reads (Drábková et al., 2002; Ribeiro & Lovato, 2007; Tarieiev et al., 2011; Drábková, 2014; Höpke et al., 2018; Kurt et al., 2022; Quatela et al., 2023).

Considering that the molecular laboratory was established at the State Museum of Natural History of the NAS of Ukraine in 2024, it was decided to test the protocols of DNA isolation and amplification on the material stored at the institutional herbarium with the acronym LWS. The herbarium LWS is one of the oldest and richest in Ukraine, hosting ca. 120,000 specimens of vascular plants and ca. 26,000 specimens of non-vascular plants (Novikov et al., 2024). However, for many years, the specimens at LWS were regularly exposed to high temperatures (ca. 90 °C) during anti-fungal and anti-insect treatment, significantly increasing the chances of DNA degradation and, therefore, the possibility of poor amplification success (Staats et al., 2011; Forrest et al., 2019). Meanwhile, there was already reported success in CTAB-based isolation and 5S rDNA intergenic spacer amplification using the material stored at the LWS herbarium (Tynkevich et al., 2022).

Höpke & Albach (2018) demonstrated that column-based DNA extraction from the herbarium material generally results in higher DNA yield and purity. They also pointed

out that column-based DNA extraction is preferred for work with herbarium specimens as it requires less sampling material and consequently causes less damage to the collection. Hence, here we share our pilot experience on column-based DNA extraction and amplification of four nuclear and five plastid regions, based on the example of 63 specimens of vascular plants (56 stored at the herbarium LWS and seven controls – see Appendix A).

Material and methods

The leaf fragments ca. 0.5–1 cm² were sampled from randomly selected herbarium specimens representing different species and collected at various years (Appendix A). Additionally, seven positive controls were implemented – six silica-dried and one herbarized (without heating) leaf samples of *Staphylea pinnata* L. (Staphyleaceae) collected in 2023.

The total DNA was isolated in July–August 2024 using the Macherey-Nagel NucleoSpin Plant II kit following a slightly modified protocol. Leaf samples were ground in the ceramic mortars with a direct addition of 500 µL of PL1 lysis buffer. Homogenizing the material would have been problematic without adding the lysis buffer to the mortar. Moreover, due to the small amount of the sampled material, taking it out from the mortar in case of dry homogenization would be problematic, too – the homogenate stuck to the mortar walls. The resulting suspension has been carefully transferred (avoiding macro fragments) to a new tube with the addition of 10 µL of RNase A solution and vortexed thoroughly. Then, the mixture was incubated at 65° for 30 minutes in a thermoshaker. Further steps followed the standard protocol. The concentration and purity (260/230 and 260/280 ratios) of total DNA have been measured spectrophotometrically using DeNovix DS-11 FX spectrophotometer/fluorometer. After that, eluted DNA was diluted 10 times and stored in the fridge until further processing.

Different regions of eluted DNA were amplified in Applied Biosystems 2720 thermal cycler using four nuclear and five plastid primers (Table 1). Thermo Scientific DreamTaq Green PCR Master Mix (2X) has been applied

Table 1. Applied primers and amplification programs.

Marker	Primer name	Primer sequence	Expected product length, bp	Reference to the primer description	Amplification program	Reference to the amplification program description
Nuclear						
ITS2	UniPlantF	TGTGAATTGCARRATYCMG	300–350	Moorhouse-Gann et al., 2018	15 min 95 °C + 40 cycles [30 s at 95 °C, 30 s at 56 °C, 1 min at 72 °C] + 10 min at 72 °C + ∞ at 10 °C	Moorhouse-Gann et al., 2018
	UniPlantR	CCCCGHYTGAYYTRGGTCDC				
ITS2	ITS2F	ATGCCGATACTTTGGTGTGAAT	400–500	Chen et al., 2010	5 min at 94 °C + 40 cycles [30 s at 94 °C, 30 s at 56 °C, 45 s at 72 °C] + 10 min at 72 °C + ∞ at 10 °C	Yao et al., 2010
	ITS3R	GACGCTTCTCCAGACTACAAT				
ITS (ITS1+ITS2)	ITS-u1	GGAAGKARAAGTCGTAACAAGG	750–2000	Cheng et al., 2016	4 min at 94 °C + 34 cycles [30 s at 94 °C, 40 s at 55 °C, 1 min s at 72 °C] + 10 min at 72 °C + ∞ at 10 °C	Cheng et al., 2016
	ITS-u4	RGTTTCTTTTCTCCCTCCGCTTA				
ITS1	ITS1	TCCGTAGGTGAACCTGCGG	400–1200	White et al., 1990	4 min at 94 °C + 34 cycles [30 s at 94 °C, 40 s at 55 °C, 1 min s at 72 °C] + 10 min at 72 °C + ∞ at 10 °C	Cheng et al., 2016
	ITS2	GCTGCGTTCCTTCATCGATGC				
Plastid						
matK	3F_KIM_f	CGTACAGTACTTTTGTGTTTACGAG	500–900	Kusia et al., 2021	5 min at 94°C + 35 cycles [30 s at 94 °C, 1 min at 52 °C, 1 min at 72 °C] + 10 min at 72 °C + ∞ at 10 °C	López et al., 2022
	1R_KIM_r	ACCCAGTCCATCTGGAAATCTTGGTTTC				Loera-Sánchez et al., 2020
rbcLa	rbcLa-F	ATGTCACCACAAAACAGAGACTAAAGC	500–650	Loera-Sánchez et al., 2020	5 min at 94 °C + 35 cycles [40 s at 94 °C, 1 min at 55 °C, 40 s at 72 °C] + 10 min at 72 °C + ∞ at 10 °C	Loera-Sánchez et al., 2020
	rbcLa-R	GTAAAAATCAAGTCCACCRCG				
trnL	c (A49325) forward	CGAAATCGGTAGACGGTACG	250–800	Taberlet et al., 1991	10 min at 95 °C + 35 cycles [30 s at 95 °C, 30 s at 50 °C, 2 min at 72 °C] + 10 min at 72 °C + ∞ at 10 °C	Taberlet et al., 2007
	d (B49863) revers	GGGGATAGAGGGGACTTGAAC				
trnL P6 loop	g (A49425) forward	GGGCAATCCTGAGCCAA	10–150	Taberlet et al., 2007	10 min at 95 °C + 35 cycles [30 s at 95 °C, 30 s at 55 °C, 30 s at 72 °C] + 10 min at 72 °C + ∞ at 10 °C	Taberlet et al., 2007
	h (B49466) revers	CCATTGAGTCTCTGCACCTATC				
trnH-psbA	psbA3_fv2	GTTATGCATGAACGTAAYGCTC	200–500 (950)	Loera-Sánchez et al., 2020	5 min at 94°C + 50 cycles [40 s at 94 °C, 1 min at 54 °C, 40 s at 72 °C] + 10 min at 72 °C + ∞ at 10 °C	Loera-Sánchez et al., 2020
	trnHf_05v2	GCRTGGTGGATTCAACAATCC				

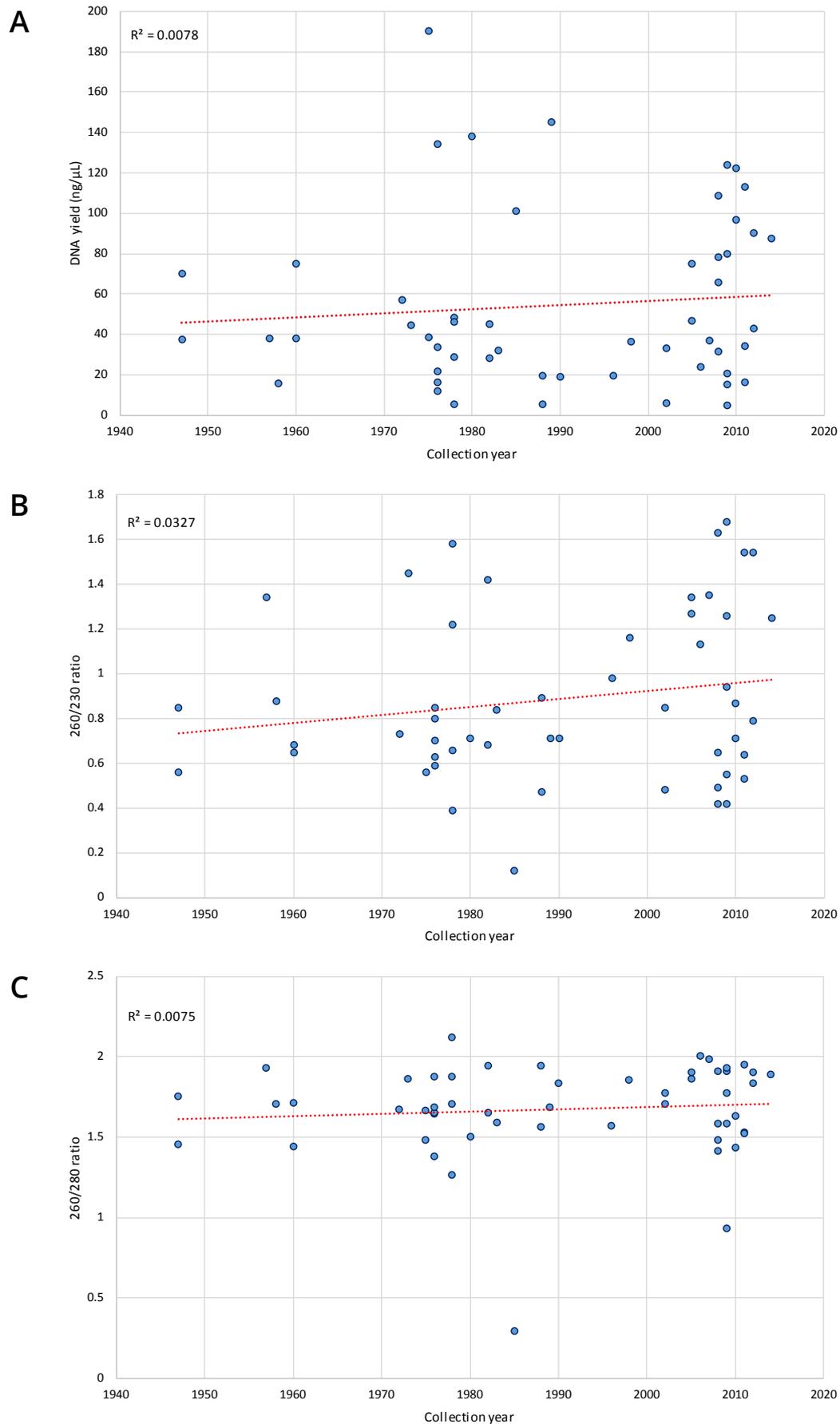


Figure 1. The dependence of DNA yield (A) and purity (B, C) from the collection year. The control samples and three outlet samples representing 1853, 1904, and 1906 years are excluded from the graphs for better visual representation.

to prepare the samples for amplification. The amplification success has been evaluated by electrophoresis realized in agarose gel prepared with $\times 10$ TBE buffer and stained by BentoLab GelGreen nucleic acid stain. The electrophoresis has been run on BentoLab portable PCR workstation at 50 V for 30 minutes.

The statistics have been performed in Microsoft Excel 2016 and Past 4.14 (Hammer et al., 2001) environments.

Results and discussion

Due to the limitation of the sample number and size, we were not able to statistically assess the full range of plant families and collection years, as well as other factors that could influence the result. The studied specimens do not allow delimiting any significant difference between the samples regarding the DNA yield (mean \pm standard deviation – 56.47 ± 43.92 ng/ μ L; coefficient of variation – 77.79%) and purity (discussed further). There was only a slightly insignificant trend in increasing DNA yield and purity jointly with collection year (Fig. 1). Regression analysis proved insignificant dependence between the collection year

and DNA extraction characteristics. Similar to our results, the DNA yield did not show a significant dependence from the collection year during the application of CTAB DNA isolation protocol and column-based DNA isolation (with the same NucleoSpin Plant II mini kit) applied for herbarium material in other studies (Höpke & Albach, 2018; Höpke et al., 2018). However, these conclusions and our outcomes contradict the reports of Zeng et al. (2018) and Marinček et al. (2022), who applied column-based isolation kits (Tiangen DNasecure Plant Kit and Qiagen DNeasy Plant Mini Kit, respectively) and noticed a significant decrease in DNA yield with samples age. In our case, the absence of advances in the DNA yield is probably caused by intense thermal treatment over the years, which smooths out the difference between more recent and older specimens at the LWS herbarium.

Nevertheless, some authors (Höpke & Albach, 2018; Höpke et al., 2018; Marinček et al., 2022) assumed that column-based DNA isolation is preferred, resulting in relatively better DNA yield and purity and is easier to handle. Marinček et al. (2022) noticed that despite the better general performance of specialized ancient DNA isolation protocol, it finally resulted in sequences comparable in

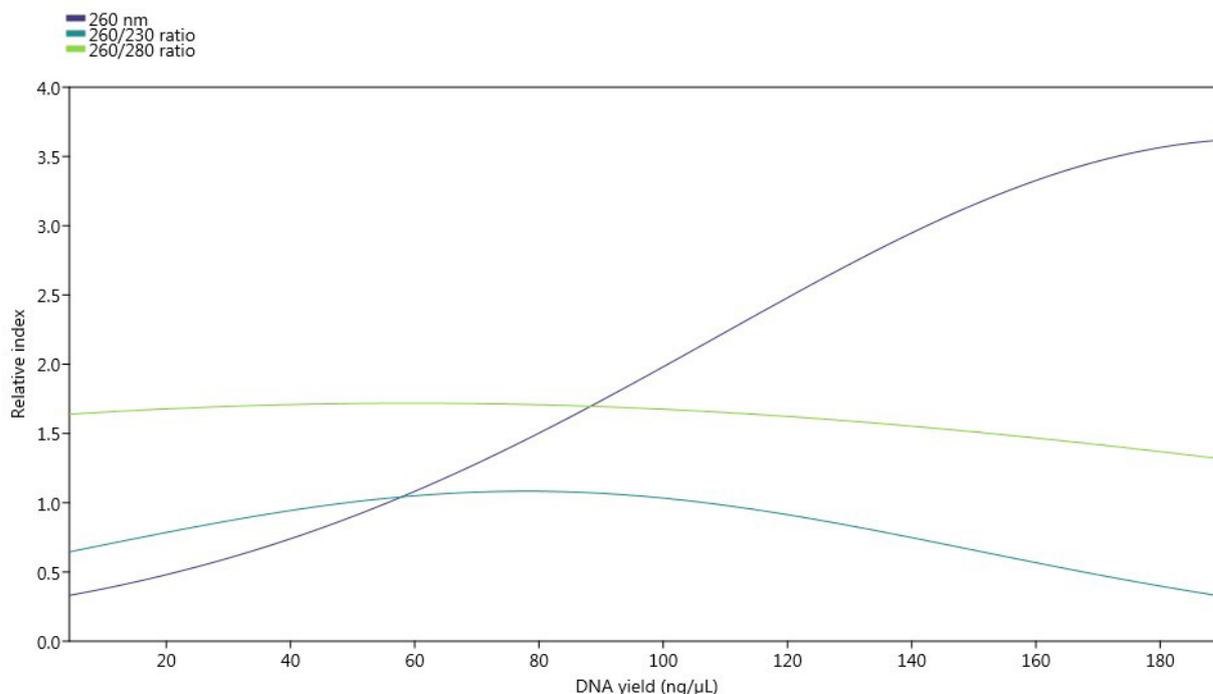


Figure 2. The dependence of DNA purity from the DNA yield. Calculated as Gaussian function in the nonlinear regression module. Initial estimation of optimum and tolerance based on the weighted average, followed by a nonlinear optimization by the Levenberg-Marquardt method in Past 4.14.

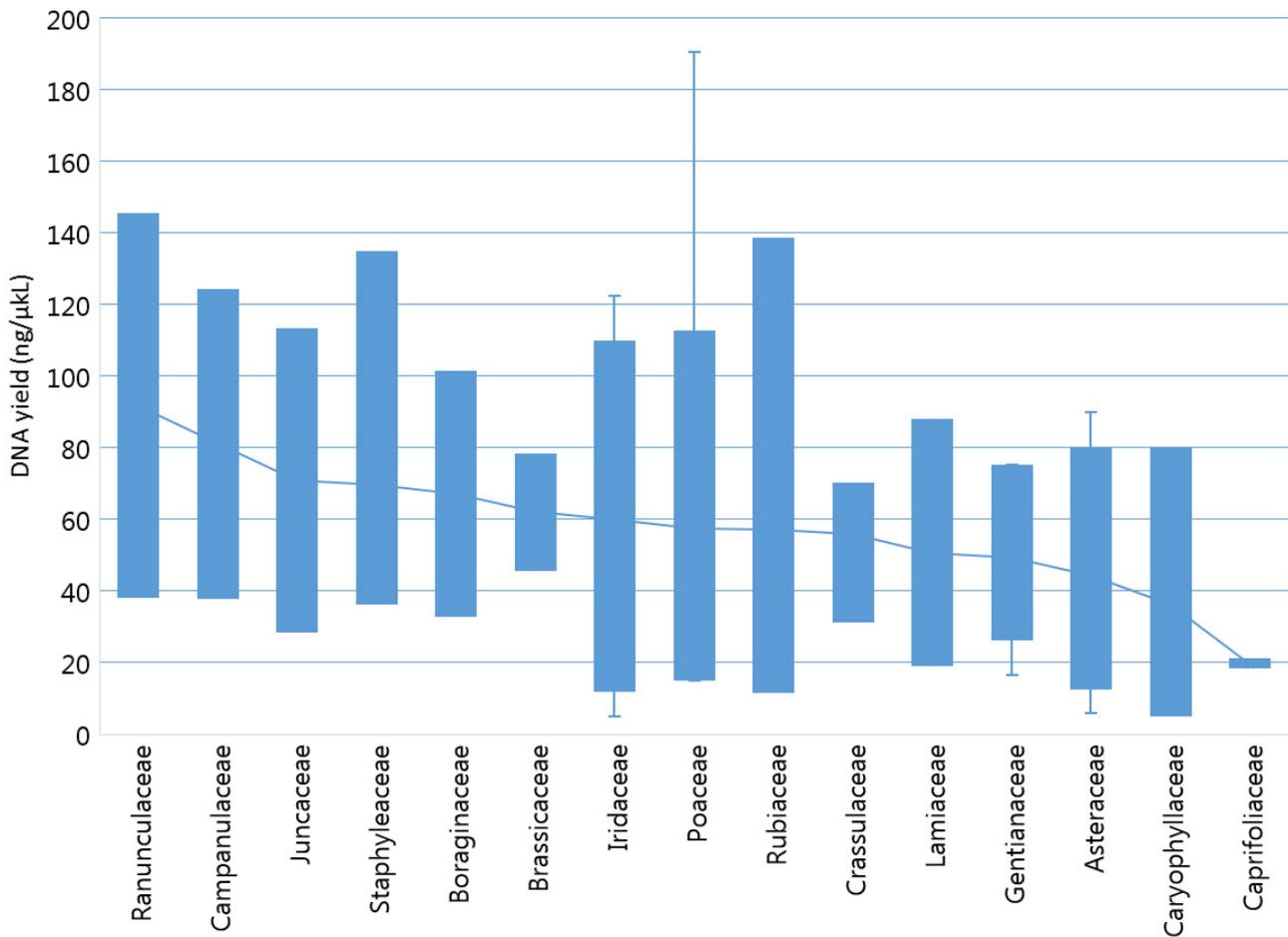


Figure 3. The boxplot of DNA yield from specimens of different plant families. The families represented by single specimen and control samples are excluded from the graph.

quality with those produced by DNA isolated with a column-based kit.

Interestingly, the best DNA purity has been achieved at a concentration diapason of ca. 50–90 ng/μL (Fig. 2). After that, the ratios 260/230 and 260/280 decreased, which can be explained either by optimal DNA concentrations in this diapason or technical peculiarities of the spectrophotometer and should be furtherly inspected. In most cases, obtained 260/230 values were markedly lower than 2.0 (mean±standard deviation – 0.85±0.36; coefficient of variation – 42.47%; Fig. 1B), which indicates contamination despite the application of the column-based DNA isolation technology. Such 260/230 values are close to those obtained as a result of CTAB DNA isolation (Höpke & Albach, 2018; Höpke et al., 2018; Kurt et al., 2022; Xie et al., 2023) and column-based DNA isolation Marinček et al. (2022) without additional purification. The observed 260/280 values were below normal

level but still near 1.8 units (mean±standard deviation – 1.66±0.36; coefficient of variation – 17.00%; Fig. 1C).

In our study, the dependence of DNA yield from the plant family of studied specimens appeared insignificant. The samples of Ranunculaceae specimens demonstrated the highest mean value of DNA yield, and those of Caprifoliaceae demonstrated the lowest. However, the standard deviation was too high for most analyzed families (Fig. 3). No particular dependence on the PCR success from the plant family or collection year was observed.

The drying method used in the herbarium technique was reported to significantly affect the DNA yield and PCR success rate (Särkinen et al., 2012; McAssey et al., 2023). In our study, silica-dried control specimens demonstrated 87.0–94.4% of successful DNA amplification. Recently collected herbarium material (one year stored control specimen)

Table 2. Successful DNA amplification with different markers applied.

Primers applied	Marker type	Successfully amplified (number of included controls)	Unsuccessfully amplified (number of included controls)	Total success rate (with controls), %	Success rate (without controls), %
<i>trnL</i> P6 Loop (g - h)	plastid	58 (7)	5	92.1	91.1
ITS1 (ITS1 - ITS2)	nuclear	24 (6)	39 (1)	38.1	32.1
ITS (ITS1+ITS2) (ITS-u1 - ITS-u4)	nuclear	23 (6)	40 (1)	36.5	30.4
ITS2 (ITS2F - ITS3R)	nuclear	23 (5)	40 (2)	36.5	32.1
<i>trnH-psbA</i> (psbA3_fv2 - <i>trnHf_05v2</i>)	plastid	23 (5)	40 (2)	36.5	32.1
<i>rbcLa</i> (F - R)	plastid	20 (6)	43 (1)	31.7	25.0
<i>trnL-F</i> (c - d)	plastid	18 (5)	45 (2)	28.6	23.2
<i>matK</i> (3F_KIM_f - 1R_KIM_r)	plastid	15 (5)	48 (2)	23.8	17.9
ITS2 (UniPlantF - UniPlantR)	nuclear	14 (7)	49	22.2	12.5

also showed nearly identical PCR success compared to silica-dried ones with all applied markers. At the same time, the success of DNA amplification for old herbarium material varied significantly and demonstrated 12.5–91.1% of successful DNA amplification (Table 2). Some of the applied markers performed better in the sense of DNA amplification success. The *trnL* P6 Loop primers demonstrated the best performance. However, application of this marker results in extremely short DNA product lengths (ca. 100 bp). Such short fragments are hard for further processing (i.e., purification with standard protocols and further sequencing) and, in most cases, allow identifying the specimens only to the genus or family level (Taberlet et al., 2007). Surprisingly, tested nuclear DNA markers showed relatively high amplification success. Among plastid markers, only *trnH-psbA* demonstrated similar performance. Despite the high amplification success reported by Moorhouse-Gann et al. (2018), UniPlant primers demonstrated the weakest result among tested ITS primers. Hence, *trnL* P6 Loop and UniPlant primers cannot be recommended for work with the herbarium material.

Conclusions

1. It was shown that tested column-based DNA isolation protocol could be successfully applied to the herbarium

material. However, the low purity of the total DNA samples obtained should be considered.

2. Tested primers (except for *trnL* P6 Loop and UniPlant) and amplification programs showed their reliability and can be recommended for work with herbarium material.
3. DNA amplification success depends on the applied markers rather than the collection year.
4. Nuclear markers generally outperformed plastid ones in work with LWS herbarium material, demonstrating better amplification success.

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Appendix A. Studied herbarium material at the LWS herbarium.

Nr	LWS accession Nr / field Nr	Family	Species / subspecies	Collection year	Preservation method
1	070459	Apiaceae	<i>Bupleurum tenuissimum</i> L.	1906	pressed and dried
2	021825	Asparagaceae	<i>Muscari botryoides</i> (L.) Mill.	1853	pressed and dried
3	114876	Asparagaceae	<i>Scilla kladnii</i> Schur	2009	pressed and dried
4	017053	Asteraceae	<i>Achillea oxyloba</i> (DC.) Sch.Bip. subsp. <i>schurii</i> (Sch.Bip.) Heimerl	1976	pressed and dried
5	095416	Asteraceae	<i>Centaurea maramarosiensis</i> (Jáv.) Czerep.	2002	pressed and dried
6	116027	Asteraceae	<i>Doronicum carpaticum</i> (Griseb. & Schenk) Nyman	1978	pressed and dried
7	117159	Asteraceae	<i>Leucanthemum rotundifolium</i> (Waldst. & Kit.) DC.	2012	pressed and dried
8	117383	Boraginaceae	<i>Pulmonaria rubra</i> Schott subsp. <i>filarszkyana</i> (Jáv.) Domin	2002	pressed and dried
9	077525	Boraginaceae	<i>Symphytum cordatum</i> Waldst. & Kit.	1985	pressed and dried
10	119944	Brassicaceae	<i>Arabidopsis neglecta</i> (Schult.) O'Kane & Al-Shehbaz	1978	pressed and dried
11	113215	Brassicaceae	<i>Arabidopsis thaliana</i> (L.) Heynh.	2008	pressed and dried
12	114577	Campanulaceae	<i>Campanula serrata</i> (Kit. ex Schult.) Hendrych	2009	pressed and dried
13	092030	Campanulaceae	<i>Phyteuma vagneri</i> A.Kern.	1960	pressed and dried
14	118938	Caprifoliaceae	<i>Scabiosa lucida</i> Vill. subsp. <i>barbata</i> Nyár.	1990	pressed and dried
15	115638	Caprifoliaceae	<i>Scabiosa lucida</i> Vill. subsp. <i>barbata</i> Nyár.	2009	pressed and dried
16	116693	Caryophyllaceae	<i>Sabulina pauciflora</i> (Kit.) A.V.Novikov	2006	pressed and dried
17	114685	Caryophyllaceae	<i>Silene nutans</i> L. subsp. <i>dubia</i> (Herbich) Zapal.	2009	pressed and dried
18	007155	Caryophyllaceae	<i>Silene zawadskii</i> Herbich	1978	pressed and dried
19	113561	Crassulaceae	<i>Rhodiola rosea</i> L.	2008	pressed and dried
20	113460	Crassulaceae	<i>Sempervivum carpathicum</i> Wettst. ex Prodan subsp. <i>carpathicum</i>	2008	pressed and dried
21	043251	Crassulaceae	<i>Sempervivum globiferum</i> L. subsp. <i>preissianum</i> (Domin) M.Werner	1947	pressed and dried
22	119472	Cyperaceae	<i>Carex curvula</i> All.	1976	pressed and dried
23	116914	Gentianaceae	<i>Gentiana laciniata</i> Kit. ex Kanitz	2012	pressed and dried
24	116676	Gentianaceae	<i>Gentiana lutea</i> L. subsp. <i>lutea</i>	2005	pressed and dried
25	116786	Gentianaceae	<i>Gentiana punctata</i> L.	2007	pressed and dried
26	116362	Gentianaceae	<i>Swertia perennis</i> L. subsp. <i>perennis</i>	2011	pressed and dried
27	074413	Gentianaceae	<i>Swertia punctata</i> Baumg.	1960	pressed and dried
28	112773	Iridaceae	<i>Crocus banaticus</i> J.Gay	1972	pressed and dried
29	115481	Iridaceae	<i>Crocus heuffelianus</i> Herb.	2010	pressed and dried
30	112026	Iridaceae	<i>Gladiolus imbricatus</i> L.	1988	pressed and dried
31	110149	Iridaceae	<i>Iris graminea</i> L.	1988	pressed and dried
32	115689	Iridaceae	<i>Iris sibirica</i> L.	2010	pressed and dried

Appendix A. Continued.

Nr	LWS accession Nr / field Nr	Family	Species / subspecies	Collection year	Preservation method
33	116180	Juncaceae	<i>Juncus bulbosus</i> L.	2011	pressed and dried
34	016776	Juncaceae	<i>Luzula alpinopilosa</i> (Chaix) Breistr. subsp. <i>obscura</i> S.E.Fröhner	1978	pressed and dried
35	112663	Lamiaceae	<i>Thymus alternans</i> Klokov	1973	pressed and dried
36	120097	Lamiaceae	<i>Thymus jankae</i> Čelak.	2014	pressed and dried
37	016733	Lamiaceae	<i>Thymus pulcherrimus</i> Schur	1996	pressed and dried
38	116659	Linaceae	<i>Linum extraaxillare</i> Kit.	2005	pressed and dried
39	073630	Oleaceae	<i>Syringa josikaea</i> J.Jacq. ex Rchb.	1982	pressed and dried
40	081841	Orobanchaceae	<i>Euphrasia tatrae</i> Wettst.	1957	pressed and dried
41	112713	Plantaginaceae	<i>Plantago atrata</i> Hoppe subsp. <i>carpathica</i> (Soó) Soó	1982	pressed and dried
42	010385	Poaceae	<i>Festuca amethystina</i> L. subsp. <i>orientalis</i> Krajina	1958	pressed and dried
43	114720	Poaceae	<i>Festuca porcii</i> Hack.	2009	pressed and dried
44	112311	Poaceae	<i>Poa granitica</i> Braun-Blanq. subsp. <i>disparillis</i> Nyár.	1983	pressed and dried
45	012597	Poaceae	<i>Poa rehmannii</i> (Asch. & Graebn.) K.Richt.	1904	pressed and dried
46	110476	Poaceae	<i>Sesleria bielzii</i> Schur	1975	pressed and dried
47	116702	Poaceae	<i>Sesleria heufleriana</i> Schur subsp. <i>heufleriana</i>	2011	pressed and dried
48	119608	Ranunculaceae	<i>Ranunculus carpaticus</i> Herbich	1975	pressed and dried
49	104365	Ranunculaceae	<i>Ranunculus malinovskii</i> Elenevsky & Derv.-Sokol.	1989	pressed and dried
50	113245	Rosaceae	<i>Rosa canina</i> L.	2008	pressed and dried
51	017272	Rubiaceae	<i>Galium transcarpaticum</i> Stojko & Tasenk.	1976	pressed and dried
52	017273	Rubiaceae	<i>Galium transcarpaticum</i> Stojko & Tasenk.	1976	pressed and dried
53	088718	Rubiaceae	<i>Galium transcarpaticum</i> Stojko & Tasenk.	1980	pressed and dried
54	063138	Staphyleaceae	<i>Staphylea pinnata</i> L.	1947	pressed and dried
55	063180	Staphyleaceae	<i>Staphylea pinnata</i> L.	1976	pressed and dried
56	107564	Staphyleaceae	<i>Staphylea pinnata</i> L.	1998	pressed and dried
57	UA01-20dr	Staphyleaceae	<i>Staphylea pinnata</i> L.	2023	pressed and dried
58	UA01-20Si	Staphyleaceae	<i>Staphylea pinnata</i> L.	2023	silica-dried
59	UA01-12	Staphyleaceae	<i>Staphylea pinnata</i> L.	2023	silica-dried
60	UA01-17	Staphyleaceae	<i>Staphylea pinnata</i> L.	2023	silica-dried
61	UA01-18	Staphyleaceae	<i>Staphylea pinnata</i> L.	2023	silica-dried
62	UA02-09	Staphyleaceae	<i>Staphylea pinnata</i> L.	2023	silica-dried
63	UA02-14	Staphyleaceae	<i>Staphylea pinnata</i> L.	2023	silica-dried

Перший прогрес у виділенні та ампліфікації ДНК з матеріалу, що зберігається у гербарії LWS

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Протестовано виділення ДНК із гербарних зразків, що зберігаються у гербарії LWS (Державний природознавчий музей НАН України, Львів, Україна) за протоколом на основі силікагелевих колонок. Виділена ДНК була ампліфікована з використанням різних ядерних і пластидних праймерів. Вихід отриманої сумарної ДНК не показав істотної залежності від року збору та родин, до яких належали зразки. Загалом, ДНК, отримана із зразків гербарію LWS, мала помірний вихід (середнє значення – 56.47 нг/мкл), але відносно низьку чистоту (середнє значення співвідношення 260/230 – 0,85 і середнє значення співвідношення 260/280 – 1,66). Успіх ампліфікації ДНК старого гербарного матеріалу коливався від 12.5 % до 91.1 % залежно від використаних праймерів. Праймери *trnL* P6 Loop продемонстрували найбільшу ефективність (91.1 % успішної ампліфікації), але через короткі фрагменти отриманої ДНК не вдалося очистити продукт для подальшої обробки. Праймери UniPlant продемонстрували найгірші результати, і лише матеріал 12.5 % досліджених зразків гербарію LWS (за винятком контрольних), був успішно ампліфікований. Загалом, ядерні праймери, за винятком UniPlant, продемонстрували кращу успішність ампліфікації (середнє значення – 31.5 %) при роботі зі зразками з гербарію LWS. В той же час, пластидні праймери, за винятком *trnL* P6 Loop, показали дещо нижчу успішність ампліфікації (середнє значення – 26.8 %).

Ключові слова: гербарні зразки, штрихкування рослинної ДНК, методи екстракції ДНК, деградована ДНК, гербарій LWS